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Novel fluoridated silk fibroin/ TiO_2 nanocomposite scaffolds for bone tissue engineering

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ABSTRACT

It is known that Fluoride ions strongly affect bone mineralization and formation. In the present study, the engineered bone tissue scaffolds are fabricated using silk fibroin (SF) and flouridated TiO_2 nanoparticles. TiO_2 nanoparticles are modified by fluoride ions, and different levels (0, 5, 10, 15 and 20 wt%) of the fluoridated TiO_2 nanoparticles (TiO_2-F) were subsequently added to the SF matrix through phase separation method to prepare silk fibroin/flouridated TiO_2 nanocomposite scaffolds (SF/TiO_2-F). Phase structure, functional groups, morphology and mechanical properties of the obtained scaffolds were evaluated by X-ray diffraction method (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and compressive testing, respectively. In vitro degradation studies of scaffolds were performed by incubating the samples in phosphate buffered saline (PBS) at 37 °C and pH 7.4 for 30 days. Additionally, the bioactivity of scaffolds was estimated in a simulated body fluid (SBF) buffered at 37 °C and pH 7.4 for 28 days. Moreover, MTT assay was used to confirm the biocompatibility of the scaffolds using human like SaOS-2 osteoblast cell line for 1, 3 and 5 days. The obtained results indicated that the mechanical properties of scaffolds have been improved by increasing the TiO_2-F amount up to 15 wt%. However, a detrimental effect was observed by a further increase in the TiO_2-F content. The bioactivity of SF/TiO_2-F nanocomposite scaffolds was promoted by flouridation of TiO_2. Furthermore, cell cytotoxicity results demonstrated that the SF/TiO_2-F nanocomposite scaffolds are nontoxic to osteoblasts. The cell fixation results after 3 days of incubation revealed that the cell attachment and spreading on SF/TiO_2-F nanocomposite scaffolds are improved with respect to SF/TiO_2 nanocomposite scaffolds control sample.

1. Introduction

Bone is a natural nanocomposite tissue [1,2]. It has the structural support role in a human body and any bone defects greatly influences the quality of life [3]. Damaged living tissues can be replaced with in vitro grown ones by tissue engineering [4,5]. Different fabricating methods have been investigated for bone tissue engineering such as solvent casting, particulate leaching, solid free form, phase separation, freeze drying, etc. to prepare porous scaffolds and improve scaffold-tissue interactions [6,7]. Porous scaffolds are able to transport the cells and growing factors for tissue regeneration. In addition, the architectures, features and microscopic direction of human tissues can be mimicked for tissue formation. Therefore, the mechanical properties, biocompatibility, and bioactivity of the scaffolds should be optimized to improve bone tissue regeneration [8,9]. Indeed, the porosity, interconnectivity and good mechanical properties of bone scaffolds provide a reliable support for bone formation [10–13].

Silk fibroin (SF) is a biocompatible core of structural protein fiber coated with sericin that can be extracted from *Bombyx mori* cocoon. This natural material is vastly used in the textile industry and medicine. Furthermore, SF has excellent cytocompatibility and is considered to be promising as a biomaterial in tissue engineering [14–16]. It has been reported that immersing SF in methanol or ethanol leads to the α-helix structure transform into a β-sheet one and make it insoluble in water. This transformation is responsible for the improvement in its mechanical properties [17].

Recent studies have shown that organic–inorganic composites have better mechanical properties as well as improved biocompatibility and

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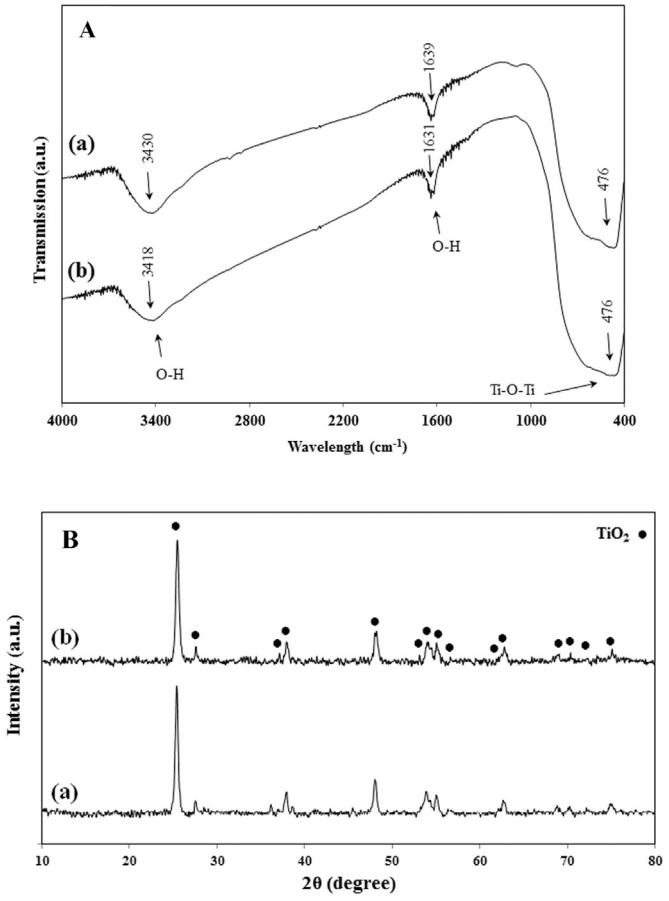

Fig. 1. A) FT-IR spectra of a) TiO_2 and b) TiO_2-F, and B) XRD patterns of a) TiO_2 and b) TiO_2-F nanoparticles.

bioactivity. Indeed, composite systems combine the advantages of polymers and ceramics to improve the properties of each component to better fit practical applications [18–23]. Currently, nanocomposites composed of biopolymeric matrices and nano-fillers are introduced as appropriate alternatives for conventional biomaterials (e.g. dense ceramic biomaterials and metallic orthopaedic biomaterials) to be used as 3D scaffolds for cell culture or bone regeneration [24–28].

Titanium dioxide (TiO_2) nanoparticles are best known as a biocompatible, bioactive, osteogenic and photostable material [29–35]. Moreover, TiO_2 scaffold are capable of inducing bone formation from the surrounding tissue and rehabilitating bone defects [10–13].

In addition, the surface chemistry and topography influence the formation of the bone to implant interface [36–39]. The implant surface can be subsequently modified to improve the biological response at the bone–implant interface [40–44]. In this regard, fluoride surface modified TiO_2 layer have better osteogenic properties, at the bone–implant interface [45–49]. **Tiainen** et al. [13] reported that the bone formation on the surface has been improved when fluoride modified titanium dioxide is used. Fluoride ions have a stimulatory action on osteogenic cells and accelerate bone regeneration [46,50–52].

The aim of this study is to prepare a novel nanocomposite scaffolds consisting of SF and TiO_2 nanoparticles and carefully characterize its properties. Furthermore, the effect of modification by fluoride ions (SF/TiO_2-F) is investigated comprehensively, thorough in vitro bioactivity tests and examining biocompatibility properties of scaffolds. Finally, the results for unmodified SF/TiO_2 nanocomposite scaffolds are presented for comparison.

2. Materials and methods

2.1. Preparation of SF/TiO_2-F nanocomposite scaffolds

Silk fibroin (SF) solution (2% w/v) was extracted using the previously reported method [53].TiO_2 nanoparticles (NanoAmor, USA) were cleaned with 1 M NaOH (Merck, Germany) solution and washed using deionized water. Fluoride modification of TiO_2 nanoparticles was performed by immersing nanoparticles in 2.0 vol% HF (Merck, Germany) for 120 s [13]. Afterwards, various amounts of TiO_2-F (0, 5, 10, 15 and 20 wt%) were sonicated for 10 min to disperse nanoparticles uniformly. Then, the prepared SF solution was added to the TiO_2-F suspension. SF/TiO_2-F solutions were subsequently placed into 24-well polystyrene plates and frozen at − 20 °C for 4 h and for further 2 h at − 80 °C. The iced SF/TiO_2-F nanocomposite scaffolds were freeze dried overnight to produce a porous matrix. These porous matrices were immersed in methanol (Merck, Germany, 99.9%) for 1 h. Immersion in methanol would induce the crystallization process and initiated the α-helix to β-sheet structural transformation. The β-sheet structure is insoluble in water. Insoluble SF/TiO_2-F nanocomposite scaffolds were prepared by removing methanol and further freeze-drying. Moreover, SF/TiO_2 nanocomposite scaffolds were prepared containing different amounts of TiO_2 nanoparticles (0, 5, 10, 15 and 20 wt%) according to our previous work [54] to compare the bioactivity and biocompatibility behaviours of SF/TiO_2 and SF/TiO_2-F nanocomposite scaffolds.

2.2. Phase structure analyses

The phase structure analyses of SF/TiO_2-F nanocomposite scaffolds with different amounts of TiO_2-F were performed using a STEO-D 64295 X-ray diffractometer (XRD, STEO, Germany) operating at 40 kV and 40 mA. All scans extended from 10 to 80° in 2θ range by Cu-Kα radiation.

2.3. Investigation of functional groups and structure of scaffolds

The functional groups of TiO_2, TiO_2-F nanoparticles and obtained SF/TiO_2-F nanocomposite scaffolds were analysed by Fourier transform infrared spectroscopy (FT-IR, MB series, ABB Bornem, Canada) in a mid-IR spectrum range within the range of 400–4000 cm^{-1}.

2.4. Morphology studies

Morphologies of the obtained SF/TiO_2-F porous structures were evaluated using Scanning Electron Microscopy (SEM, TESCAN, Czech Republic).

2.5. Porosity measurements

The porosity percentage of scaffolds was measured according to the Archimedes' Principle [55,56], and determined as follow:

$$\text{porosity}\ (\%) = \frac{(w_2 - w_3 - w_s)/\rho_e}{(w_1 - w_3)/\rho_e} \quad (1)$$

where w_S is the weight of the dry scaffold; w_1, w_2 and w_3 are the weight of bottle filled with ethanol, bottle containing ethanol and scaffold, and bottle taken out of ethanol-saturated scaffold, respectively. ρ_e is the density of ethanol.